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Mutagenicity Studies and Risk Estimation
of Complex Mixtures of Organic Airborne Contaminants

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Typical human exposure to atmospheric pollutants rarely involves only a single chemical. Rather, most natural atmospheres contain complex mixtures of such potentially dangerous chemicals as polycyclic aromatic hydrocarbons (PAHs), nitro pyrenes and oxygenated hydrocarbons. Although the risk associated with individual chemicals can be assessed, the risk due to mixtures is much more difficult to anticipate since the components may interact in several different and unpredictable ways. Our general approach to this problem has been to measure the mutagenicity of both individual chemicals and defined mixtures of the same chemicals. From these data we hope to identify basic principles of chemical interaction, as they relate to human risk, in natural atmospheres.

The research reported here involves two groups of chemicals: Benzo(a)pyrene (BaP), 7,12-Dimethyl benz(a)anthracene (DMBA), 1-Nitro pyrene and (11) Benzo(b)fluoranthene, Dibenz(a,h)anthracene and 1,1,1-Trichloroethane. All have been tested alone or in combinations in two short term bioassays; the Ames *Salmonella* assay and the *in vivo* murine bone marrow micronucleus assay.

The data obtained suggest some basic principles concerning complex atmospheric mixtures and indicate where further testing is required.

Materials and Methods

- a) Chemicals: BaP, DMBA, 1-Nitropyrene, Dibenz(a,h)anthracene, Benzo(b)fluoranthene, and 1,1,1-Trichloroethane were obtained from Aldrich Chemical Company.
- b) Bacterial Strains: Histidine auxotrophs of Salmonella typhimurium strains TA 98 (frame shift mutant), and TA 100 (substitution deletion strain) were obtained from Professor B. Ames.
- c) Mice: B6C3F₁ male mice, were purchased from Harlan Sprague Dawley Inc. The mice were used at 8-9 weeks of age.
- d) Ames' Salmonella assay: The procedure described by Maron and Ames (1983) was followed. The "Plate incorporation test" was used for mutagenicity testing. All the mutagens were tested with and without S9. Mutagenicity, in units of revertants/ μ g, was calculated from the initial linear portion of the dose (μ g/plate) response curve.

If the solvent control value was within normal range, a test chemical or mixture that produced a positive dose response over three concentrations, with the highest increase equal to at least twice the solvent control value for TA 98 and TA 100, was considered mutagenic.
- e) In vivo bone marrow micronucleus assay: This assay measures chromosomal breakage in bone marrow cells. The assay procedure used was that of Heddle and Salamone (1981) which is a modification of the original procedure of Schmid (1973, 1975).

Results

a) Ames Assay

(i) Group I tested individually.

BaP, DMBA, 1-Nitropyrene are mutagenic to strains TA 98 and TA 100. However, BaP and DMBA are mutagenic only with S9 metabolic activation, whereas 1-Nitropyrene is mutagenic even in the absence of S9 metabolic activation.

(ii) Group II tested individually.

Neither Benzo(b)fluoranthene nor Dibenz(a,h)anthracene is mutagenic to strain TA 98 in the presence or absence of activation.

(iii) Group I tested in combinations.

Pairwise combinations of Group I and all three tested together produce more complicated responses which are shown in Table I. Tests of Group II in combinations have not yet been completed.

b) Micronucleus Assay

All chemicals were tested over dose ranges from approximately 10 mgm/kg to several hundred mgm/kg. (The actual doses depended on the previously measured LD₅₀). All samples were collected and assayed 48 hr after injection. The data obtained are shown in Table 2. In Group I BaP and DMBA were mutagenic and 1 Nitropyrene weakly mutagenic. BaP and DMBA combined show less than additive. In Group II Benzo(b)- fluoranthene and Dibenz(a,h)anthracene are mutagenic while 1,1,1-Trichloroethane is not. All combinations of Group II show less than additive effects.

TABLE 1.

Ames assays of potential atmospheric pollutants

Chemical(s) tested	S9	Mutagenicity with		Remarks
		TA 98	TA 100	
<u>GROUP I</u>				
A. BaP	(- (+)	- +	- +	
B. DMBA	(- (+)	- +	- +	
C. 1-Nitropyrene	-	+	+	Direct acting mutagen
(A + B)	+	+	+	(1)
(A + C)	-	+	+	(2)
(B + C)	-	+	+	(3)
(A + B + C)	-	+	+	(4)

GROUP II

D. Benzo(b)-fluoranthene	(- (+)	- -	NT NT	
E. Dibenzo(a,h)-anthracene	(- (+)	- -	NT NT	
F. 1,1,1-Trichloroethane		NT	NT	

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NT = not tested

- (1) Less than additive with TA 98. Synergistic with TA 100.
 (2) Synergistic with both tester strains.
 (3) Synergistic with both tester strains.
 (4) Less than additive with both tester strains.

TABLE 2.

Micronucleus assay of potential atmospheric pollutants

Chemical(s) Tested	Mutagenicity
<u>GROUP I</u>	
A. BaP	Positive
B. DMBA	Positive
C. 1-Nitropyrene	Very weakly mutagenic
(A + B)	Less than additive
(B + C)	NT
(A + C)	NT
(A + B + C)	NT
<u>GROUP II</u>	
D. Benzo(b)fluoranthene	Positive
E. Dibenzo(a,h)anthracene	Positive
F. 1,1,1-trichloroethane	Negative
(D + E)	Less than additive
(D + F)	Less than additive
(E + F)	Less than additive
(D + E + F)	Less than additive

=====

NT = not tested

Discussion

The preliminary data presented here demonstrate many of the difficulties encountered when one is trying to assess the biological risk of complex atmospheric mixtures. Such problems include; mutagenicity in one test system but not another (Benzo(b)fluoranthene and Dibenzo(a,h)anthracene), synergism with one tester strain in the Ames assay and inhibition with another, the requirement for activation in one test system but not another, etc. Although much of the data is preliminary, when it is considered in conjunction with other testing of PAHs in our



laboratory (Raj et al., 1988) some initial observations appear. For example, (1) Pairwise assays rarely give additive responses in the test systems we have used. (2) Pairwise combinations of a promutagen and a direct acting mutagen usually produce a synergistic response in the Ames Assay. Such a response is achieved even in the absence of S9 although it is even higher in the presence of S9. (3) Most synergistic responses are in the range of 1.5 to 3.0 X the additive responses. (4) The micronucleus assay rarely exhibits an additive or synergistic response.

Clearly it is premature to suggest broad principles of pollutant interaction, however the results of our experiments encourage us that such principles exist and await only further research to reveal them.

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